Universidade Federal de Ouro Preto
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Born Into the Wild: O que formas jovens de Copepoda podem nos dizer sobre a qualidade ambiental?

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Resumo

A abundância de formas jovens de copépodos (náuplios e copepoditos) foi analisada como indicador de qualidade ambiental em ecossistemas do Baixo Rio Doce (rios, lagos, lagoas), submetidos a diferentes tipos de impactos (eutrofização e rejeito de mineração de ferro). A influência sazonal também foi considerada na variação temporal da composição e abundância das formas jovens. Condições de eutrofia e hiper-eutrofia foram observadas para os sistemas lacustres, com alguns desvios sazonais do estado trófico. Não houve distinção da concentração de metais entre os diferentes tipos de ambientes, no entanto, os lagos apresentaram maior concentração de Al, Ba, Mn e Zn, enquanto as concentrações de Fe caracterizaram as águas do Rio Doce. A abundância de náuplios e copepoditos foi maior nos lagos profundos, indicando que esses sistemas lacustres mais estáveis são importantes sítios reprodutivos de copépodos no Baixo Rio Doce. A maior abundância de náuplios de copépodos durante o período chuvoso pode estar associada à maior variação do estado trófico dos ambientes, favorecendo a diversificação de alimentos e a reprodução das formas adultas. Náuplios estiveram sempre negativamente correlacionados com metais, indicando maior sensibilidade à presença desses elementos na água. Os resultados obtidos confirmam que a inclusão das formas jovens de copépodos em análises ecológicas representa uma importante ferramenta de descrição da qualidade ambiental de ecossistemas aquáticos. A significância ecológica das formas jovens de copépodos deveria ser considerada em protocolos de impacto ambiental.

Palavras-chave

Copepoditos, Náuplios, Metais, Eutrofização, Impactos, Baixo Rio Doce

Abstract

The abundance of young forms of copepods (nauplii and copepodites) was analyzed as an indicator of environmental quality in ecosystems of the Lower Rio Doce (rivers, lakes, lagoons), subjected to different types of impacts (eutrophication and mining waste). Seasonal influence was also considered in the temporal variation in the composition and abundance of young forms. Conditions of eutrophy and hyper-eutrophy were found for lake systems, with some seasonal deviations in trophic state. Metals concentrations were not distinct between the environments, but shallow lakes presented highest concentrations of Al, Ba, Mn and Zn, while Fe concentrations characterized the Doce River waters. The abundance of nauplii and copepodites was greater in the deep lakes, indicating that these more stable lake systems are important reproductive sites for copepods in the Lower Rio Doce. The greater abundance of copepod nauplii during the rainy season may be associated with greater variation in the trophic state of the environments, favoring the diversification of food and the reproduction of adult forms. Nauplii were always negatively correlated with metals, indicating greater sensitivity to the presence of these elements in the water. The results obtained confirm that the inclusion of young forms of copepods in ecological analyzes represents an important tool for describing the environmental quality of aquatic ecosystems. The ecological significance of young forms of copepods should be considered in environmental impact protocols.

Key words

Copepodits, Nauplius, Metals, Eutrophication, Impacts, Lower Doce River

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Introduction

The rapid deterioration of the environmental quality of continental aquatic ecosystems has been described in Brazil and worldwide (Reid et al., 2018; Moi et al., 2022), with significant negative impacts on aquatic biodiversity and the provision of ecosystem goods and services. Intensive use of agricultural fertilizers, urban expansion, and the intrusion of organic waste into water bodies can accelerate the process of eutrophication, as waste and agrochemical products are continuously released into the environment (Torremorell et al., 2021). In the Lower Doce River region (Espírito Santo), in addition to the impact of eutrophication on some water bodies (Coimbra; Alcântara; de Souza Filho, 2021), the iron mining tailings from the Fundão dam collapse in November 2015 drastically altered the Doce River's fluvial environment and associated lacustrine ecosystems (Pauly et al., 2024). The impacts of mining tailings on various components of the freshwater trophic chain have already been recorded, including metal bioaccumulation (Zebral et al., 2022), malformations in fish larvae (Bonecker et al., 2019), effects on periphyton species composition (Zorzal-Almeida and Fernandes, 2021), and a reduction in zooplankton species diversity (Santos et al., 2022), among others.

An important tool for monitoring the impacts of anthropogenic actions on aquatic ecosystems is environmental bioindicators—organisms that respond in a short period to changes in the aquatic ecosystem (Stankovic, 2013). The zooplankton community has been used as a sensitive and reliable descriptor of environmental impacts, especially those resulting from human activities. Features such as rapid response to environmental variations and a short life cycle are fundamental to the effectiveness of using zooplankton organisms as bioindicators (Perbiche-Neves et al., 2021).

In general, almost all studies on the bioindication of zooplanktonic microcrustaceans focus on the abundance of adult forms, mainly due to the greater ease of taxonomic identification. Many authors consider copepods to be the most abundant metazoans on Earth (Schminke, 2007; Herstoff et al., 2023). Their importance as fundamental intermediate links in the planktonic grazing chain in aquatic environments, in energy transfer (Turner, 2004), in the biogeochemical cycle (Elser et al., 1988), and as food for fish larvae (Fernando, 1994), makes them key elements for understanding the changes occurring in aquatic ecosystems.

Before reaching adulthood, copepods undergo a complex cyclomorphosis, with four developmental stages (egg, nauplius, copepodite, and adult), considered by some authors as the most complete example of metamorphosis among Crustacea (Dahms, 1992). The transition between copepod developmental stages is accompanied by drastic changes in morphology, as well as behavioral and physiological adaptations to explore different ecological niches, particularly in relation to the type and size of the ingested food.

Thus, the completion of the copepod development cycle is intrinsically related to the availability of a wide range of resources, which involves not only the available food but also limnological conditions, the fulfillment of nutritional requirements, and the occupation of the water column, determined by their ability to move and escape predation.

Copepods are highly sensitive to environmental changes and drastic alterations in water quality, including those caused by seasonal variations, which may affect their life history strategies and the development of juvenile stages such as nauplii and copepodites (Kiorboe and Sabatini, 1995). Seasonal variations in water quality may influence reproductive behavior and the development of copepods, particularly their juvenile stages. Increases in turbidity, hydrodynamic movements, and the runoff of pollutants from the watershed can hinder food encounters and reduce primary production (Aranguren-Riaño et al., 2011), with decisive consequences for herbivorous zooplanktonic forms, such as most freshwater copepods.

The relevance of different growth phases of freshwater copepods in environmental quality assessments has generally been neglected in zooplanktonic studies. In this paper, we explore how different growth phases of copepods (nauplii and copepodites) can serve as good predictors of environmental quality in aquatic ecosystems affected by different anthropogenic impacts (eutrophication and the influence of mining tailings). We test the following hypotheses: (i) the abundance of juvenile forms (nauplii and copepodites) will be lower during the rainy season due to increased turbidity and metal concentrations in the water across the three types of environments (Doce River, lakes, and ponds); (ii) the distribution of nauplii, Cyclopoida copepodites, and Calanoida copepodites abundance is regulated by different abiotic variables (limnological descriptors, trophic state index, and metal concentration) across different seasonal periods, independent of the type of environment, and can serve as an indicator of the environmental quality of the studied ecosystems.

Materials and Methods

Study Area

The studied environments are located in southeastern Brazil, in the state of Espírito Santo, encompassing the Lower Doce River region (sampling points in Linhares, Regência, Itapina, Resplendor, and the river mouth), the deep lakes Nova, Juparanã, and Palmas (> 3 meters depth), and the shallow lakes Cacimbas, Monsarás, and Areal (< 3 meters depth). These lakes are situated around agricultural, pasture, and urban areas, with evidence of eutrophication detected in Juparanã and Areal (Salinas et al., 2020). The deep lakes Juparanã and Nova show signs of thermal stratification, exhibiting a monomictic mixing pattern, with stratification during the warm and wet season and mixing during the dry and cool season (Bozelli et al., 1992; Gonçalves et al., 2016). These lakes are connected to the Doce River by a drainage network, which can reverse flow direction under flood conditions. Both deep and shallow lakes are subject to fluvial intrusion during flooding events (RRDM, 2019).

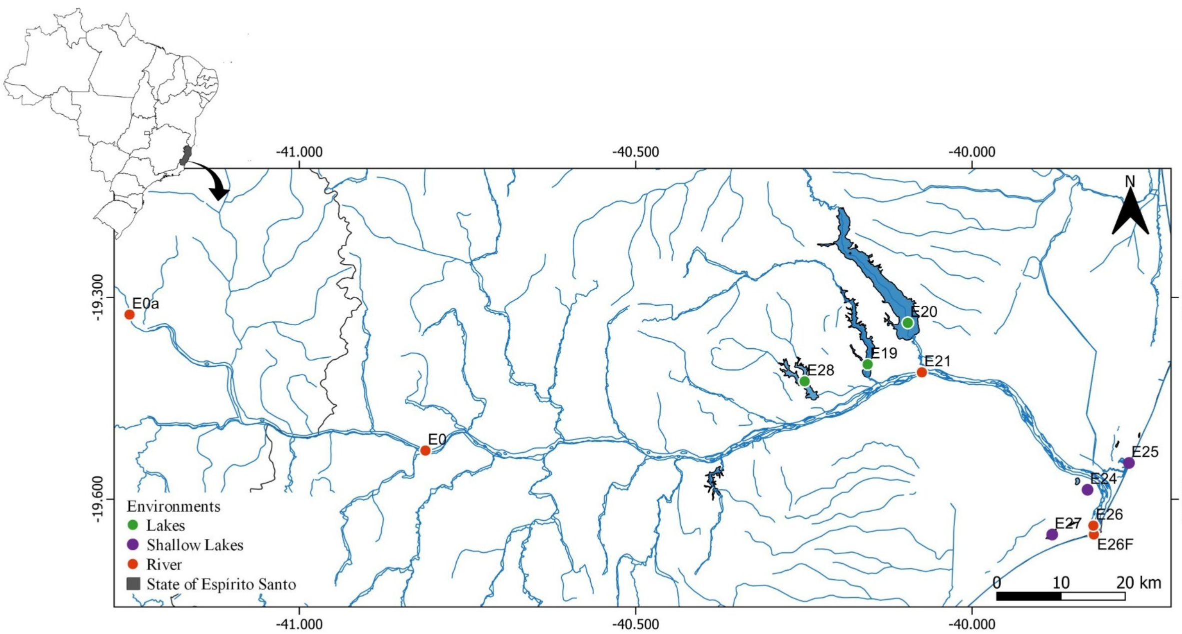

Figure 1: Location of sampling points. E19 - Nova Lake, E20 - Juparanã Lake, E21 - Doce River (Linhares), E24 - Areal Lake, E25 - Monsarás Laguna, E26 - Doce River (Regência), E27 - Cacimbas Lake, E28 - Palmas Lake, E0 - Doce River (Itapina), E0a - Doce River (Resplendor), E26F - Doce River (mouth).

Sampling and laboratory analysis

Water and zooplankton samples were collected monthly from August 2022 to January 2024 from the surface of the water column. Limnological variables, such as water temperature (°C), dissolved oxygen (mg/L), and turbidity (NTU), were measured using a Horiba U-53 multiparameter probe. Water samples were collected with a 3 L Van Dorn bottle to analyze additional limnological variables and metal concentrations according to the following methodologies: chlorophyll a ($\mu g.L^{-1}$) (Jespersen & Christoffersen, 1987), total phosphorus (TP) ($\mu g.L^{-1}$) and total nitrogen (TN) ($\mu g.L^{-1}$) (Valderrama, 1981), and total fractions of iron, aluminum, barium, manganese, zinc, and vanadium (Fe, Al, Ba, Mn, Zn, and V, respectively) (all in $\mu g.L^{-1}$) (Lim et al., 2017).

Zooplankton samples were collected by filtering 50 L of surface water through a 68 µm mesh net. Before preservation in a 4% buffered formaldehyde solution, organisms were anesthetized with carbonated water. Three aliquots of each qualitative and quantitative sample were analyzed, with at least 100 individuals observed per aliquot. Zooplankton were analyzed using a 1 mL Sedgwick-Rafter counting chamber under optical microscopy and identified to the lowest possible taxonomic level. Samples with fewer than 50 individuals were fully counted. Sorted specimens were cataloged and stored in the Aquatic Ecology, Evolution, and Conservation Laboratory (LAECO) collection at the Federal University of Ouro Preto.

Statistical Analysis

To obtain a general characterization of the environments in terms of trophic status, the Trophic State Index (TSI) was calculated using chlorophyll-a and total phosphorus values (CETESB, 2013), following the formula:

$$TSI = \frac{TSI(PT) + TSI(CL)}{2}$$

PT: total phosphorus concentration measured at the water surface, in $\mu g.L^{-1}$;

CL: chlorophyll-a concentration measured at the water surface, in $\mu g.L^{-1}$.

Samples were classified according to the Carlson Trophic State Index (1977), as modified by Lamparelli (2004), which categorizes environments as ultraoligotrophic (TSI ≤ 47), oligotrophic (47 < TSI ≤ 52), mesotrophic (52 < TSI ≤ 59), eutrophic (59 < TSI ≤ 63), supereutrophic (63 < TSI ≤ 67), and hypereutrophic (TSI > 67).

To assess the normality of the data across environments and seasons, the Shapiro-Wilk test was used. To test the first hypothesis, a Generalized Linear Mixed Model (GLMM) was applied (lmer function, R package lme4 and lmerTest). An ANOVA was performed to validate the significance of the GLMM model (Anova function, R package car). For testing the second hypothesis, Redundancy Analysis (RDA) was used, a multivariate analysis that assesses the relationship between a data matrix, such as species and environmental variables (rda function, R package vegan). Values were considered significant when $p \leq 0.05$.

Results

In terms of limnological descriptors, no differences were found for these variables between the Doce River, deep, and shallow lakes (Mann-Whitney test, $p > 0.05$) (Table 1). All studied environments were slightly acidic, with median pH values ranging from 6.2 to 7.3, with no seasonal variability. The same pattern was observed for water temperature, with median values above 27°C during both dry and rainy periods. All the shallow lakes in the study had well-mixed water bodies and were not stratified at the time of sampling, but in the deep lakes, thermal stratification was detected during the rainy period. Eutrophic conditions characterized all the lakes and the Doce River. High concentrations of nutrients, particularly nitrogen, were detectable. Total nitrogen (µg/L) ranged from 3371.6 in the shallow lakes to 1121.2 in the deep lakes, and total phosphorus concentrations exceeded 20 µg/L in all environments, reaching a maximum median value during the rainy period in the Doce River (115.6 µg/L). In terms of algal biomass (Chl-*a*), shallow lakes were the most enriched environment compared to the deep lakes and the Doce River. The median Chl-*a* value in shallow lakes reached 6.9 µg/L, while in deep lakes the mean Chl-*a* was 3.3 µg/L. In the Doce River, algal biomass was consistently lower, with a median value of 1.7 µg/L during the rainy period and 1.1 µg/L during the dry period. Metals were detected in all environments, with the highest concentrations of Al (with a maximum value of 3181.1 µg/L during the rainy period in the Doce River), Fe, Ba, and Mn. A slight trend towards higher concentrations of Al, Ba, Mn, and Zn was observed in the shallow lakes, while Fe was the predominant element in the waters of the Doce River, reaching 3427.1 µg/L during the rainy period (Table 1).

Table 1: Mean and standard deviation of limnological parameters and metals in the water of the studied environments (shallow and deep lakes, river) of the Lower Doce River, Brazil. Chl-*a*: Chlorophyll a; T (°C) = Water temperature; DO = Dissolved Oxygen; TP = Total Phosphorus; TN = Total Nitrogen.

	Deep Lake		Shallow Lake		River	
Abiotic factors	Rainy	Drought	Rainy	Drought	Rainy	Drought
Chrophyll-*a*	3.3 ± 2.3	3.9 ± 3.2	6.9 ± 5.1	4.8 ± 3.5	1.7 ± 1.8	1.1 ± 0.7
DO (mg/L)	8.6 ± 0.6	8.7 ± 1.5	6.5 ± 1.3	7.9 ± 1.6	8.1 ± 1.2	9.3 ± 0.8
pH	7.4 ± 0.3	7.1 ± 0.6	6.5 ± 0.3	6.8 ± 0.3	6.91 ± 0.2	7.2 ± 0.3
Temperature (°C)	30.5 ± 0.7	26.1 ± 1.2	29.4 ± 1.4	26.1± 1.3	29.4 ± 1.8	25.7 ± 1.5
TN (µg/L)	1121.2 ± 202.4	1057.5 ± 195.1	3371.6 ± 560.6	3337.3 ± 1.038.3	2817.4 ± 551.3	2550.8 ± 744.6
TP (µg/L)	29.3 ± 4.6	28.9 ± 15.5	28.8 ± 12.7	32.8 ± 14.5	115.6 ± 140.4	61.9 ± 74.8
Turbidity	2.8 ± 2.3	1.7 ± 1.3	65.3 ± 141.7	17.6 ± 18.6	275.3 ± 307.6	22.7 ± 17.1
Al (µg/L)	435.9 ± 253.0	338.3 ± 278.3	1823.0 ± 379.4	1025.5 ± 551.2	3181.1 ± 992.9	1837.7 ± 790.0
Fe (µg/L)	499.4 ± 258.8	338.1 ± 260.7	4485.8 ± 1109.4	1592.6 ± 435.8	3427.1 ± 940.1	1643.7 ± 647.8
Zn (µg/L)	2.7 ± 1.8	2.8 ± 1.6	10.9 ± 12.1	3.5 ± 3.2	18.9 ± 19.6	5.9 ± 9.6

Partially confirming our first hypothesis, the abundance of young individuals of copepods (nauplii and copepodites of Calanoida and Cyclopoida) was greater during the dry period in deep and shallow lakes ($p < 0.001$), but no significant difference was detected in the Doce River ($p = 0.174$) (Figure 2).

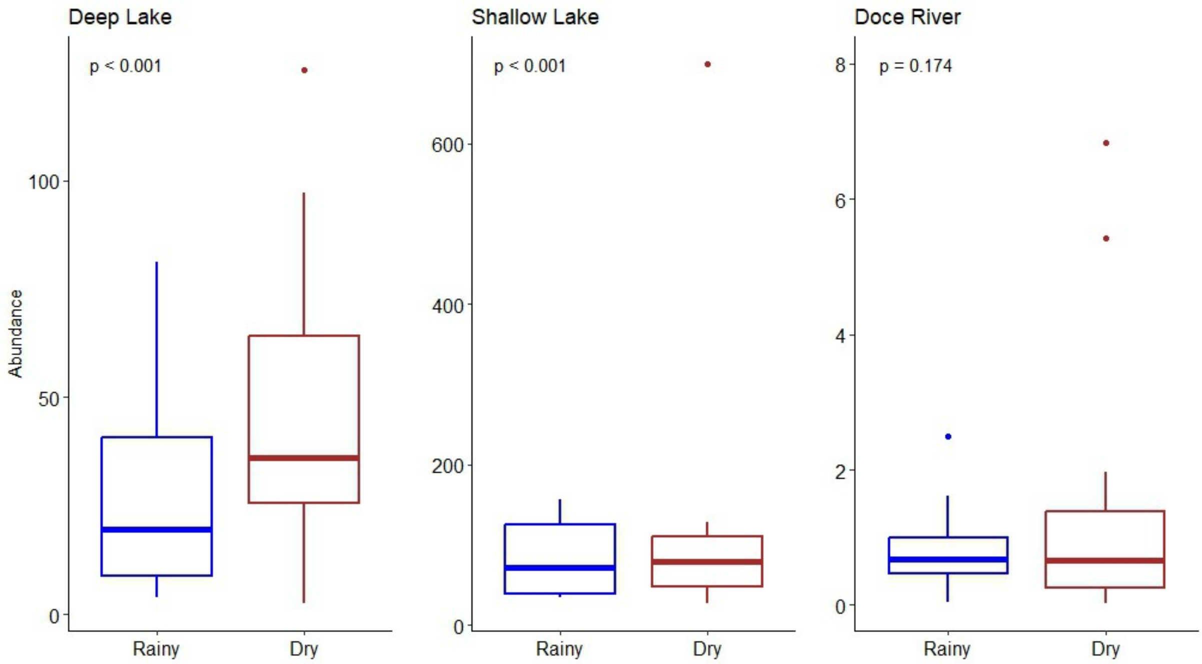

Figure 2: Box plot of the abundance of nauplii and copepodites of Copepoda (ind.L^{-1}) in the studied environments (lakes, shallow lakes, and river) and seasonal periods (rainy and dry).

The Redundancy Analysis (RDA) performed with the trophic classification and copepod nauplii and copepodites indicated that the canonical axis was statistically significant for the rainy and dry seasons (R^2 adjusted = 0.369; $p = 0.003$). Explanatory variables explained 94% of the total variance for the rainy and dry periods (Fig. 3A and B). Overall, mesotrophic and eutrophic conditions predominated in all studied environments, with oligotrophic conditions occurring only in deep lakes during the dry period (Figure 3B). For the Doce River, meso-eutrophic conditions predominated mainly during the rainy period, associated with nutrients (Total P and Total N) and metals.

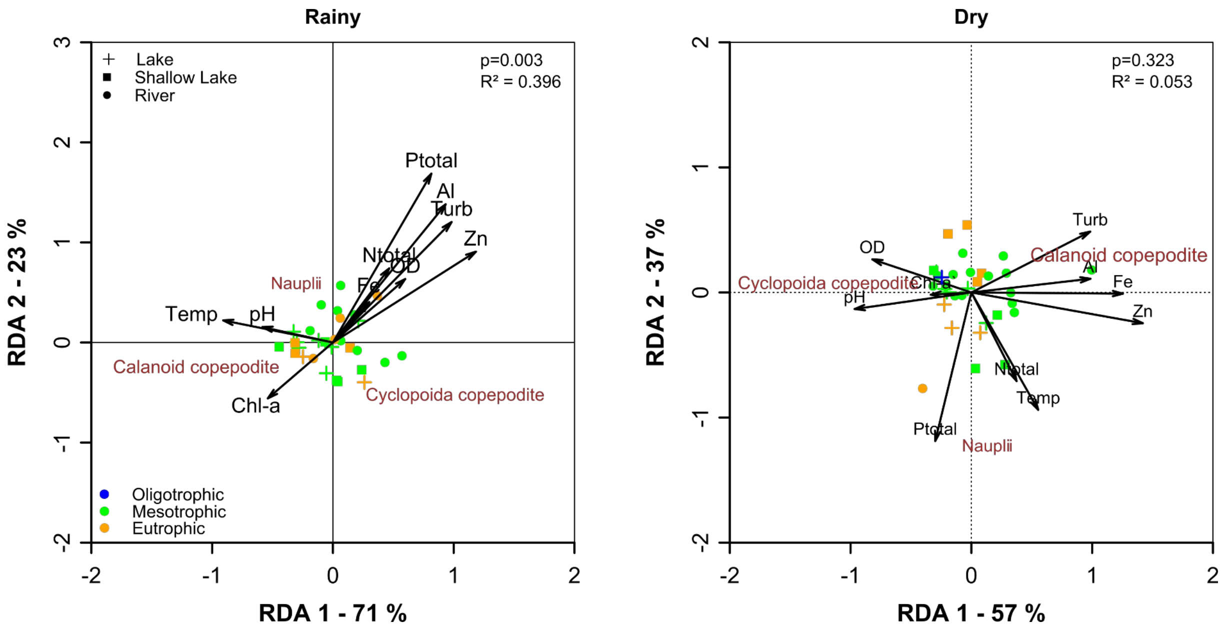

Figure 3: Redundancy Analysis (RDA) relating abiotic variables to young Copepoda (copepodites and nauplii) and the trophic states of each environment during the rainy (A) and dry (B) periods of the Baixo Rio Doce, Brazil. NTU (Turbidity), Temp (Temperature), DO (Dissolved Oxygen), Mn (Manganese), Ba (Barium), Fe (Iron), Zn (Zinc), Al (Aluminum), Chl-*a* (Chlorophyll-a), TP (Total Phosphorus).

In the rainy period, metals (Al, Fe, Zn), Total P and Total N, and dissolved oxygen were predominantly related to the Doce River, with no positive relationship to any form of young copepods (copepodites or nauplii). Copepod nauplii were positively associated with mesotrophic conditions, water temperature, and pH. Calanoid copepodites were related to Chl-*a* in the meso-eutrophic conditions of lakes and shallow lakes. In the dry period, metals, turbidity, Total N, and temperature were related to rivers and shallow lakes and favored calanoid copepods. Copepod nauplii were also associated with shallow lakes and limnological descriptors (Total N, water temperature, and Total P). Cyclopoid copepods were favored by pH and dissolved oxygen in shallow lakes with meso-eutrophic conditions and the mesotrophic status of the Doce River.

Discussion

The present study analyzed, for the first time, the effects of different environmental conditions (eutrophication and metals) on the abundance of young forms of copepods in aquatic environments of the Lower Doce River. Our results indicated a negative effect of eutrophication and contaminants associated with iron mining waste on the abundance of nauplii and copepodites of Copepoda, suggesting that the study of young forms of zooplankton can contribute to a better understanding of the environmental quality of water bodies.

Copepods are the most abundant metazoans on the planet (Raymont, 1980) and are a fundamental group associated with controlling primary production in aquatic ecosystems (Kunzmann *et al*., 2019). In addition to their crucial importance in the aquatic food chain, the complexity of their life cycle, which includes four stages (egg, nauplius, copepodite, and adult), makes them important elements in understanding environmental variations and their effects on community dynamics. Many studies on zooplankton communities do not consider the presence of nauplii, primarily due to methodological issues, as they may be undersampled using plankton nets with mesh openings greater than 100 µm. However, the traditional plankton nets used in freshwater zooplankton collections have mesh openings ranging from 50 to 68 µm, allowing for the collection of specimens from all developmental stages of freshwater copepods.

Some authors (Bottger, 1987; Roff et al., 1995) warn that zooplankton collections using nets with 64 µm mesh openings may still underestimate the abundance of nauplius and copepodite stages, although the abundance results obtained in the present study did not indicate underestimation in the collection of these vital stages.

A high abundance of nauplii and copepodites of copepods was observed in lacustrine environments (lakes and ponds) and in the channel of the Doce River. In the shallow lakes, the abundance of young forms of copepods was high (> 100 ind.L^{-1}), with a significant seasonal difference (higher during the dry period), confirming our first hypothesis regarding the seasonal effect on the abundance of young forms of copepods.

Although there are few experimental studies in Brazil on the effect of temperature on egg production and the ontogenetic development of copepods, the decisive effect of temperature seems to be consensual among researchers (Maier, 1989; Ban et al., 2000). More stable water column conditions and high temperatures are essential factors for the development of young copepod stages, especially naupliar forms, which are considered extremely vulnerable compared to copepodites, which are more resilient and have a competitive advantage in altered environments (Sell et al., 2001; Gemmell; Buskey, 2011). Furthermore, it has also been observed that the abundance of Copepoda has decreased with the gradual increase in temperature over the past decades (Rice; Stewart, 2016).

Organisms utilize the energy obtained from feeding to perform various essential functions for survival, such as growth, reproduction, evasion of predation, and obtaining food resources. However, in adverse situations, it becomes necessary to establish priorities and choose where to invest energy most effectively, a trade-off strategy (Litchman, 2013). In the occurrence of environmental disturbances, zooplankton may choose to invest more energy in reproduction than in growth, representing a survival and persistence strategy (Litchman, 2013; Kiørboe, 2024).

The abundance of nauplii appears to be directly influenced by the abundance of the adult population, but there may also be an additional source of young individuals in the population, which are the resting eggs produced under stress conditions (Grice et al., 1981; Glippa et al., 2011). No resting eggs of copepods were detected in the analyzed samples; thus, we consider that the nauplii population primarily originated from the hatching of subitaneous eggs.

Meso-eutrophic conditions predominated in all studied environments and can be considered a reflection of the intensive use of the basin, corroborating the results described in the reports of the Water Quality and Sediment Monitoring Program (PMQQS).

Environments with higher concentrations of Chl-*a*, along with temperatures that increase the proliferation of microalgae, may imply greater availability of food and nutrients, favoring recruitment (Wilson, 1973; Zhang et al., 2022; Berger; Steinberg; Tarrant, 2024). Moreover, this higher concentration of Chl-*a* may increase water turbidity, reducing predation pressure by fish (Hall, 1982; Rothschild; Osborn, 1988; Mackenzie et al., 1994; Havens and Beaver, 2011). Finally, the reproduction of Copepoda is strongly impacted by variations in pH (Kurihara et al., 2004), as the abundance of the group is compromised in acidified aquatic ecosystems (Wærvågen and Nilssen, 2010; Min et al., 2021).

In summary, the study demonstrated that the abundance of young forms is impacted by limnological descriptors, as well as seasonal variations, confirming the second hypothesis. These variations in abundance demonstrated that nauplii and copepodites are directly affected, highlighting that these organisms can be good environmental bioindicators. Therefore, the use of young forms of Copepoda to infer information about the health of aquatic ecosystems could be a promising tool for conservation. Further studies exploring these instances could significantly contribute to environmental monitoring and assessment efforts.

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